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LIGAND-EXCHANGE CHROMATOGRAPHY OF AMINO ACIDS ON NICKEL–CHELEX 100

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SUMMARY

A ligand-exchange chromatographic procedure for the selective separation of amino acids from inorganic ions is presented. It was found that the binding of amino acids to the nickel–Chelex 100 resin is pH dependent. At pH 8.5–9.1, only the basic amino acids lysine, histidine and arginine are quantitatively attached to the complex, whereas at pH 11, other amino acids with the exception of aspartic acid and glutamic acid are also bound, although not quantitatively. All of the amino acids can be eluted from the complex with 3 *M* ammonia solution without the displacement of nickel ions from the complex. This method can be used for the removal of the basic amino acids from solutions in the presence of inorganic ions as well as other amino acids.

INTRODUCTION

The principle upon which ligand-exchange chromatography is based is that a transition metal is fixed on a solid support and this solid sorbent can be used for the exchange of bound ligands of the metal. By using this system, ligands such as amino acids and amines can be removed from their medium by the formation of complexes with the metal attached to the support, and consequently water or liquid coordinates of the metal will be displaced.

In earlier procedures, Sephadex complexed with copper was used in column chromatography for the separation of amino acids from peptides under alkaline conditions[1]. Ligand-exchange chromatography on thin layers and columns of natural and substituted celluloses has also been performed by loading these supports with metals such as antimony, cobalt, mercury and silver[2]. This chromatographic procedure has also been shown to be suitable for the separation of D- and L-amino acids based on the stereoselective effects in α-amino acid–copper complexes[3].

New developments in ligand-exchange chromatography began with the use of resins with a stronger affinity for metals such as Chelex 100, which contains iminodiacetate functional groups. Thus, Siegel and Degens[4] were successful in removing amino acids from sea water by using Chelex 100 complexed with copper and claimed

that the complex was capable of removing all of the amino acids with the exception of cystine at pH 8–9 with 100% recovery. Buist and O'Brien[5] employed a similar method for the separation of amino acids from peptides in urine at pH 11 by using sodium tetraborate buffer. The separation of amino acids from oligopeptides was also achieved by Boisseau and Jouan[6], who employed copper–Chelex 100 equilibrated with ammonia solution at pH 10.3. Ligand-exchange chromatography on copper–Chelex 100 has also been used for the separation of nucleic acid components[7] and for amines, hydrazines and purine and pyrimidine bases[8]. Recently, Bellinger and Buist[9] reported that copper–Chelex 100 quantitatively binds all of the common amino acids except glutamic acid and aspartic acid, even at pH 10.0–10.2.

Although some discrepancy exists among the results reported by different investigators, we decided to use this procedure for the removal of amino acids from waste water, as we were interested in studying certain problems related to the purification of waste water and the relationship between metabolic activities of purifying bacteria and the presence of certain amino acids. We found that the binding of amino acids to copper–Chelex 100 is pH dependent* and this complex cannot bind quantitatively some amino acids, notably the dicarboxylic acids at pH 8–9, in contrast to the claims made by some investigators. For these studies, it was particularly desirable to find a technique by which one could concentrate and remove basic amino acids in the presence of other amino acids. It was therefore thought that this could be achieved by replacing copper with other transition metals that would have a weaker affinity with amino acids. In this paper, we describe the properties of nickel–Chelex 100 and its potential for ligand-exchange chromatography.

MATERIALS AND METHODS

The sodium salt of Chelex 100 resin (100 g, 100–200 mesh; Bio-Rad Labs., Richmond, Calif., U.S.A.) was exhaustively washed with double-distilled water (*ca.* 30 l) until the washing effluent gave no precipitate with silver nitrate solution (the initial wash water showed a pH of 12.0 and gave precipitation with silver nitrate). The resin was then suspended in a saturated solution of nickel chloride** with vigorous stirring at 4° for 24 h. The nickel–Chelex 100 complex was then filtered off and washed thoroughly with double-distilled water until the wash water was free from Ni^{2+} ions (detection test with Merckoquant Ni^{2+} test paper; E. Merck, Darmstadt, G.F.R.).

Chelation chromatography at slightly alkaline pH

The nickel–Chelex 100 was suspended in 3 *M* ammonia solution and stirred for 1/2 h; by this treatment, the initial green colour of the nickel–resin complex turned dark blue. Afterwards, the nickel–resin complex was filtered off, and washed successively (*ca.* 10–15 l) with double-distilled water until the washings showed a pH of 8.5–9.1.

Glass columns (20 × 1 cm) were packed with 10 ml of nickel–resin complex. A solution of the mixture of the common amino acids corresponding to 1 μmole of

* The results of these experiments will be published elsewhere.

** Nickel chloride, ammonia solution, hydrochloric acid and other reagents were reagent-grade materials obtained from E. Merck, Darmstadt, G.F.R.

each amino acid (with the exception of Trp, Gln and Asn) was added to 250–500 ml of double-distilled water (pH $\approx$ 5.5), and the solution was allowed to pass through each column at a flow-rate of about 2–2.5 ml/min. The pH of the solution after passing through the column was nearly the same as the original pH of the nickel–resin suspension. Afterwards, each column was washed with 10 ml of double-distilled water. The columns were then eluted with 50 ml of 3 or 6 *M* ammonia solution in order to remove the attached amino acids from the complex. Although nickel did not seem to be eluted with the ammonia solution, the ammonia-eluted material was passed through a second column of Chelex 100 (Na^+) so as to remove any traces of nickel that might have been displaced from the nickel–Chelex 100. It was observed that no nickel was retained by the second column, which indicated that no nickel is removed from the complex during elution with ammonia solution. The eluted ammonia solution was dried on a rotary evaporator and most of the remaining traces of ammonia were removed *in vacuo* in a desiccator in the presence of concentrated sulphuric acid. The amino acids were taken up in about 5 ml of 6 *N* hydrochloric acid and the excess acid was removed by rotary evaporation. The residue was then dissolved in citrate buffer of pH 2.2 and the amino acids were quantitatively determined by automatic high-pressure ion-exchange chromatography[10].

In the case of waste water samples, the samples were filtered through glass filter funnels and 1000 ml of the sample were allowed to pass through a 10-ml nickel–resin column. The original pH of the waste water samples was about 8. The samples were either introduced as such, or their pH was adjusted to about 5.5 by adding hydrochloric acid. It should be mentioned that these samples show a buffering capacity, which is presumably due to the presence of phosphate, borate and other anions; therefore, a relatively large amount of hydrochloric acid had to be added in order to lower the pH.

Chelation chromatography at strongly alkaline pH

After the nickel–Chelex 100 had been washed free from Ni^{2+} ions, it was suspended in phosphate buffer solution of pH 11 (50 ml of 0.05 *M* disodium hydrogen phosphate plus 4.1 ml of 0.1 *M* sodium hydroxide solution, the final volume being adjusted to 100 ml). The final pH of the suspension was adjusted to 11 by the addition of 1 *N* sodium hydroxide solution (over a period of a few hours, until no change in the pH was observed). The columns were packed as before and were further washed with a small amount of buffer. A sample of amino acids (1 μmole of each amino acid) was added to 250–500 ml of the phosphate buffer and the solution was allowed to run through the columns at a flow-rate of 2–2.5 ml/min. Blank tests were also performed by passing phosphate buffer solution alone through columns packed with nickel–Chelex 100. Elution and amino acid analysis were performed as described above.

RESULTS AND DISCUSSION

The sodium salt of Chelex 100 binds the nickel ions, but we found that there is no binding between the hydrogen form of the resin and Ni^{2+} ions. However, with copper, the binding is effected with either the hydrogen or the sodium form of the resin and it has been reported[11] that a better conversion to the copper form is achieved

by first converting the resin into the hydrogen form and subsequently reacting it with a Cu^{2+} solution.

By performing ligand-exchange chromatography on nickel–Chelex 100, it could be demonstrated that the binding of amino acids to the metal–resin complex is pH dependent. At slightly alkaline pH (8.5–9.1), only the three basic amino acids lysine, histidine and arginine were bound to the nickel–Chelex 100. These amino acids were then eluted from the complex and analyzed chromatographically. An ion-exchange chromatogram of these amino acids is shown in Fig. 1. By decreasing the loading of the amino acids on the column or increasing the amount of packing material, it became clear that 10 ml of nickel–Chelex 100 is sufficient for the sorption of 1 μmole of each of the amino acids, and no other amino acids except the three basic ones can be bound to the complex at this pH even when a much larger amount of nickel–resin is used. The removal of these amino acids from the solid sorbent can be achieved with 3 *M* ammonia solution and the use of a more concentrated solution was unnecessary. The recoveries of the amino acids were almost quantitative (95 $\pm$ 5%), allowing for analytical errors. In these experiments, it was found that nickel ions are bound so strongly to the resin that the metal was not displaced from the complex during elution with ammonia. This was the case whether solutions containing strong electrolytes such as waste water or phosphate buffer or only distilled water were passed through the columns prior to the ammonia elution. On the other hand, it has been reported[4] that slight elution of copper from copper–Chelex 100 by ammonia solution occurs if a strong electrolyte solution has been passed through the column. This observation was also confirmed by us, and copper leakage was particularly noticeable after the passage of waste water samples or buffer containing inorganic ions, but slight leakage also occurred after the passage of distilled water. Similar results have also been reported by Shimomura *et al.*[8], who observed that the metal leakage of sulphonated polystyrene resin loaded with copper(II) is much greater than when the same resin is loaded with nickel(II).

Ligand-exchange chromatography on nickel–Chelex 100 at slightly alkaline pH therefore provides a selective method for the removal of basic amino acids from

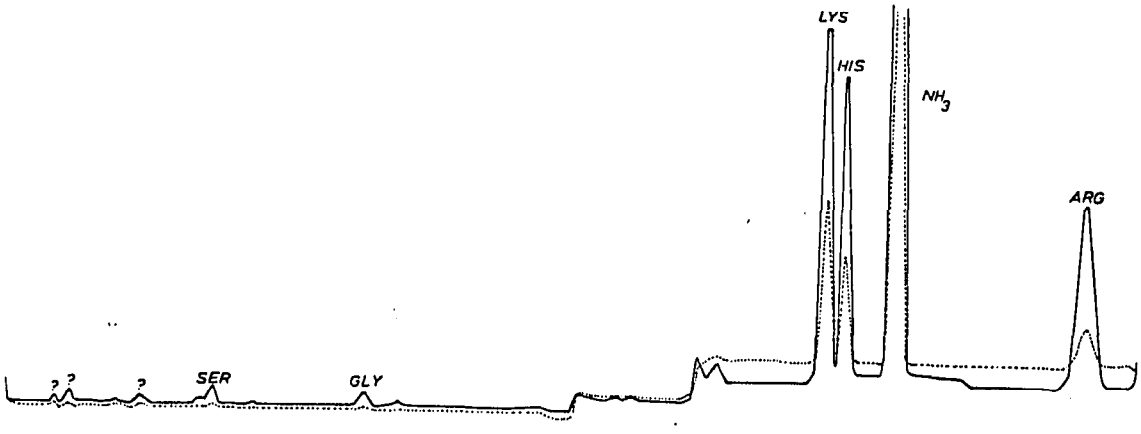

Fig. 1. Ion-exchange chromatogram of amino acids removed from a mixture of all of the common amino acids by binding to nickel–Chelex 100 at slightly alkaline pH (8.5–9.1). ———, Absorbance at 570 nm; - - -, absorbance at 440 nm.

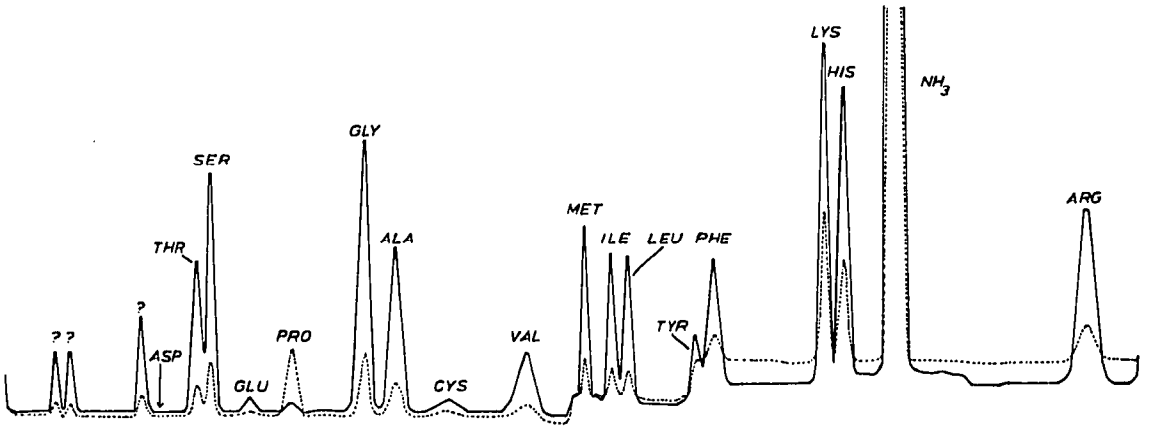

Fig. 2. Ion-exchange chromatogram of amino acids removed from a mixture of all of the common amino acids by binding to nickel–Chelex 100 at pH 11 (the recovery of each amino acid is given in Table I). ———, Absorbance at 570 nm; ---, absorbance at 440 nm.

water samples were treated for the separation of their basic amino acid content, in addition to the basic amino acids, small amounts of some other amino acids such as Ser, Gly and Ala were also attached to the polymer, owing to the high pH of waste water samples.

Chelation chromatography on nickel–Chelex 100 at strongly alkaline pH (pH 11) showed that, apart from the basic amino acids, neutral amino acids are also bound to the complex, but not quantitatively, whereby cysteine and tyrosine are least bound. The attachment of the two dicarboxylic acids aspartic and glutamic acid was negligible, as can be seen in Fig. 2. The recoveries of each amino acid in this experi-

their medium in the presence of inorganic ions and other amino acids. When waste

TABLE I

RECOVERIES AND p*I* VALUES OF AMINO ACIDS

Average rates of the sorption and recovery of amino acids removed from mixtures of the common amino acids by ligand-exchange chromatography on nickel–Chelex 100 at pH 11.

Amino acid	*Recovery (%)*	*pI*
Lysine	93	9.74
Histidine	96	7.59
Arginine	95	10.76
Aspartic acid	00	2.77
Threonine	31	—
Serine	48	5.68
Glutamic acid	2	3.22
Proline	76	6.30
Glycine	86	5.97
Alanine	51	6.00
Cysteine	17	5.07 (30°)
Valine	24	5.96
Methionine	19	5.74
Isoleucine	27	6.02
Leucine	33	5.98
Tyrosine	8	5.66
Phenylalanine	35	5.48

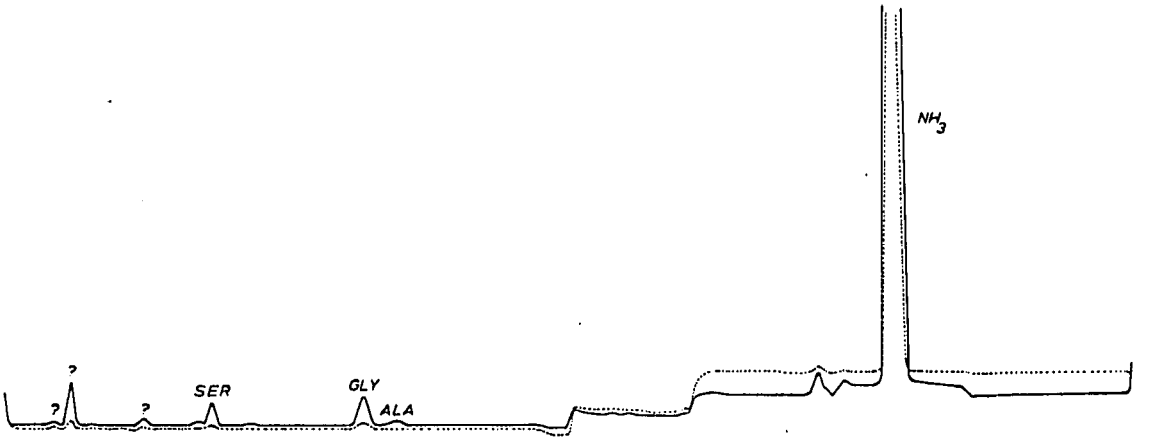

Fig. 3. Ion-exchange chromatogram of a blank test at pH 11. The first three peaks at the beginning of the run are of unknown origin and correspond to none of the common amino acids; these peaks are also present in the chromatograms in Figs. 1 and 2. ———, Absorbance at 570 nm; ---, absorbance at 440 nm.

ment are given in Table I. The pH values at the isoelectric point at 25° (p*I*) for the amino acids[12] are also given in Table I for the purpose of comparison between them and the rates of sorption. It can be seen that only the amino acids with high p*I* values (basic) are completely bound by the complex, while there is no binding of acidic amino acids (low p*I* values) to the nickel–resin, the other amino acids being intermediate.

The blank test was also performed by passing phosphate buffer through the column; the ion-exchange chromatogram of this test shows the traces of certain amino acids, and there also exist two small and one relatively larger peak at the beginning of the run, which can be attributed to imino groups released from the resin[4] (Fig. 3). These peaks also exist when solutions of amino acids were passed through the columns, as seen in Figs. 1 and 2.

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